Reduplication of Nuclear Membranes in HeLa Cells Infected with Adenovirus.* By Michael B. Gregg‡ and Councilman Morgan. (*From the Departments of Medicine and Microbiology, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York.*)§

Previous electron microscopic studies of tissue cultures infected by type 3 adenovirus have revealed clusters of parallel membranes (1), as well as intranuclear tubules composed of lamellae (2). This communication reports the appearance of multiple membranes, probably nuclear in origin, in association with type 10 adenovirus.

Adenovirus, type 10,[1] was grown in HeLa cells maintained on a medium consisting of 0.5 per cent lactalbumin hydrolysate, 0.25 per cent glucose, 0.1 per cent yeast extract, 0.3 per cent tris(hydroxymethyl) aminomethane, and 10 per cent horse serum. Infected tissues were prepared for electron microscopic study by techniques previously described (2).

Fig. 1 illustrates part of a HeLa cell nucleus containing numerous adenoviral crystals. The cell wall traverses the upper left corner. Separating the cytoplasm at the left and lower borders from the nuclear matrix are parallel membranes, which occasionally form oval diverticular structures. At the upper right and in the central lower third of the figure lie oval, homogeneous bodies. Although frequently seen in adenoviral infections, their nature is not known. An adjacent cell occupies the lower right corner.

Fig. 2 shows the upper portion of the preceding micrograph. The parallel lamellae on the left exhibit localized defects. Several lamellae appear to terminate in the cytoplasm. Within the nuclear matrix, near the upper border of the picture, are two parallel membranes (see arrow), which appear to be similar to the peripheral membranes.

In Fig. 3 the membranes are viewed at higher magnification. Cytoplasm occupies the top; nuclear matrix the bottom. On the right the membranes bulge into the cytoplasm and are indistinct. This loss of definition may represent a basic structural change or may result from superimposition of membranes lying obliquely in the section. Though seldom as extensive as the case herein illustrated, the membranes appeared as multiples of two.

In the light of the foregoing, it seemed possible that other adenoviral types might cause reduplication of membranes. Accordingly, micrographs of adenovirus types 1 to 8 were reviewed and, indeed, occasional nuclei were found to possess four peripheral membranes. The phenomenon differed quantitatively, rather than qualitatively, among the viral types. The tubules composed of lamellae arranged in concentric or spiral form and the clusters of parallel membranes, previously referred to, have so far been encountered only in nuclei infected with type 3 adenovirus. Presumably these structures represent an unusual manifestation of a propensity on the part of the nuclei to form membranes.

Other observers (3, 4) have described different but perhaps analogous lamellar systems in various cell types. These annulate lamellae were seen in the cytoplasm, frequently at some distance from the nucleus. Although annuli have not been seen in the lamellae associated with adenoviruses, the location of the membranes both at the surface and within the matrix of infected nuclei suggests that they are nuclear in origin.

In this connection it is of interest that striking multiplication of nuclear membranes has been observed in human amnionic and HeLa cells infected with herpes simplex virus (5). The appearance of intranuclear lamellae situated close to the virus, together with the presence of viral particles between the multiple nuclear membranes, suggested that the peripheral coat of the virus was formed from the lamellae, and that the laying down of membranes behind the viral particles as they approached the surface of the nucleus enabled them to emerge into the cytoplasm without loss of nuclear continuity. In the cytoplasm this virus was usually lodged within vacuoles, the walls of which appeared to be of nuclear origin. However, although adenoviral particles were seen within intranuclear laminated tubules (2), no virus was encountered between the membranes described herein. In the case of the adenoviruses, the release seems to depend upon disruption of the nucleus.

Little is yet known concerning the relation of

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nuclear and cytoplasmic membrane systems to viral replication in mammalian cells. It is possible, however, that nuclear membrane reduplication, as seen in adenoviral and herpes simplex infection, represents a basic and important type of cellular response to infection by a variety of viral agents.

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Explanation of Plates

Plate 257

Fig. 1. Part of a nucleus containing crystals of adenovirus. × 16,000.

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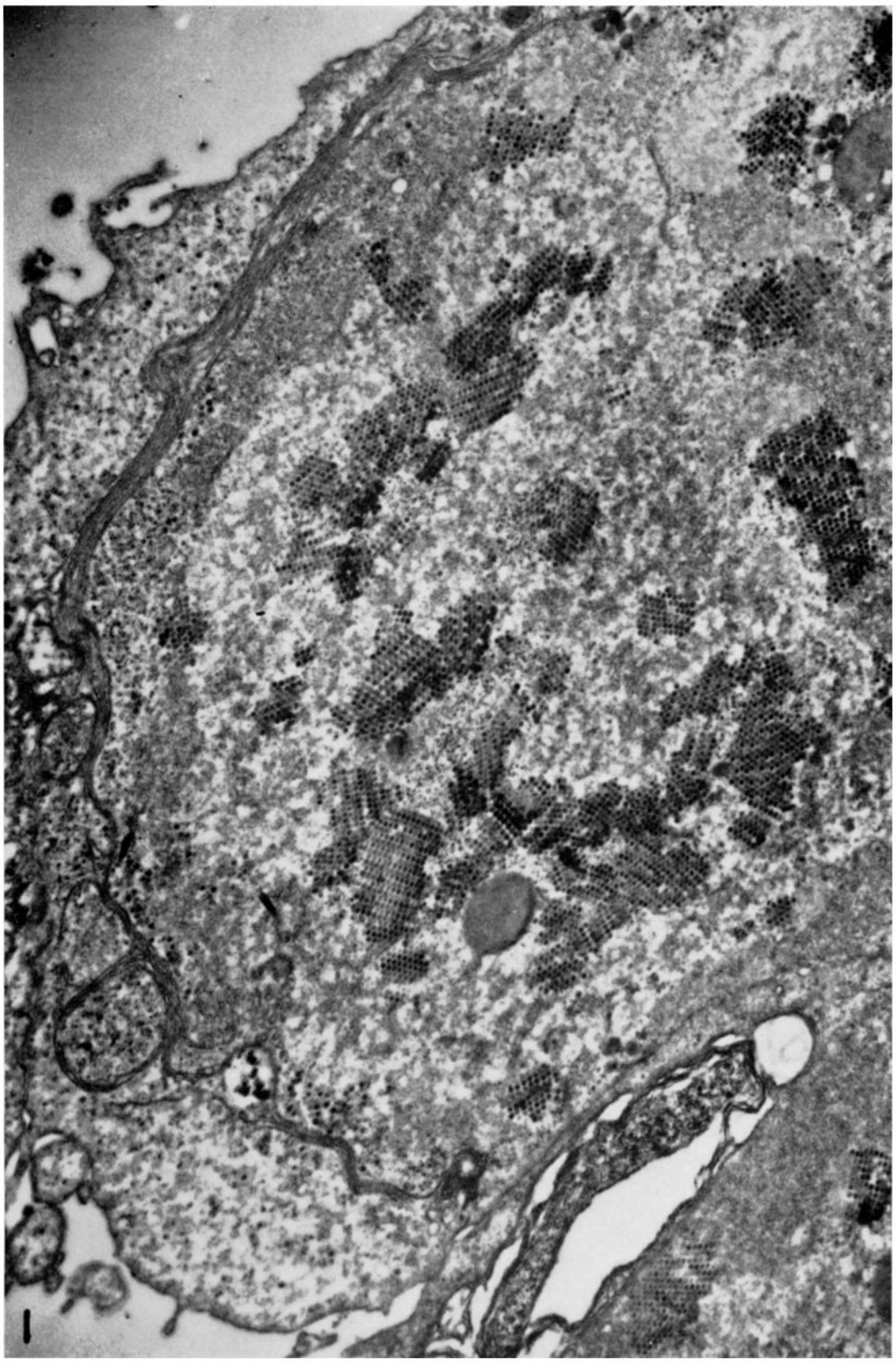

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PLATE 258

FIG. 2. The upper portion of Fig. 1 viewed at higher magnification. Several of the membranes terminate in the cytoplasm at the left. × 37,000.

FIG. 3. Multiple membranes separating the cytoplasm (above) from the nuclear matrix (below). × 44,000.

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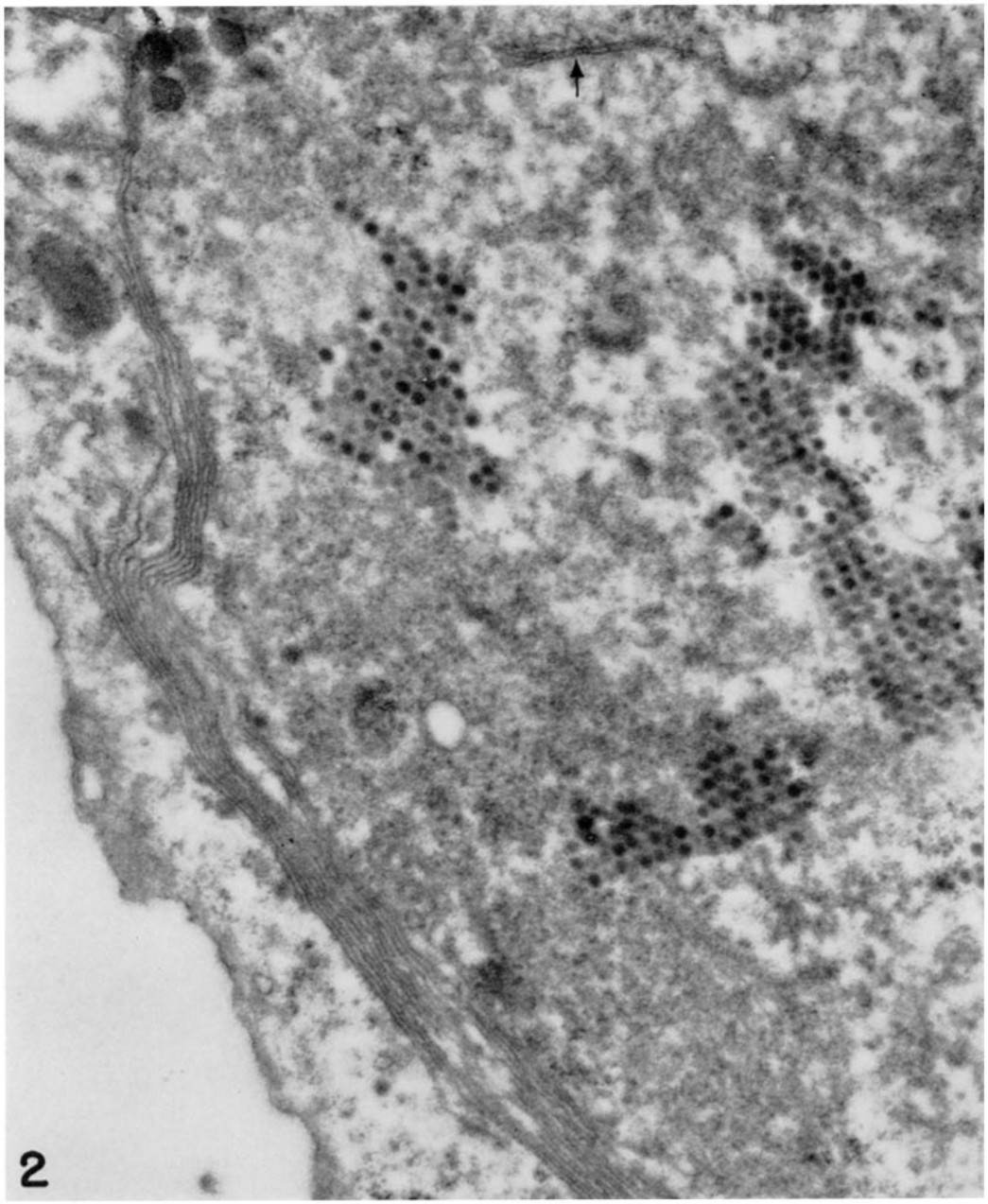

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